

# Chlorin Accumulation as a Proxy for Changes in Past Productivity in Annually Laminated Sediments of Saanich Inlet

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## Introduction

The ability to predict the future behavior of the ocean as a sink for fossil fuel CO<sub>2</sub> rests on our understanding of phytoplankton productivity in today's ocean as well as past changes in ocean production. Since the anthropogenic increase of atmospheric carbon dioxide is expected to result in substantial warming of the earth's surface (Houghton and others, 1996), understanding the interactions between climate and carbon cycling has become of considerable public and scientific interest.

The chlorophyll concentration in seawater is commonly used to estimate primary productivity in the ocean. The accumulation of chlorins, transformation products of chlorophyll, recorded in ocean sediments has been proposed as a measure for changes in total primary production over glacial-interglacial time scales (Harris et al., 1996). Developing proxies for changes in primary production over shorter time periods is also critical. The effect of interannual and interdecadal variability such as El Niño-Southern Oscillation (ENSO) and the Pacific Decadal Oscillation (PDO) on fisheries recruitment (Ware and Thomson, 1991; Mantua et al., 1997) and climate change (Ware, 1995; Zhang et al., 1996) has been established.

Aside from instrumental records, decadal records of regional climate variability in the Pacific Northwest are limited. In this paper we demonstrate that there is no evidence of degradation of chlorophyll pigments in the top 1.4 m of anoxic sediments from Saanich Inlet. Since these chloropigments are a direct result of overlying productivity, changes in the concentration of these compounds can serve as a record of variations in phytoplankton production.

## Experimental

Site Description Saanich Inlet (British Columbia, Canada; Figure 1) is a highly productive fjord characterized by a 75-m sill that limits the renewal of bottom water. This physical isolation combined with the high primary productivity in the fjord results in anoxic conditions at depths below 150m (Richards, 1965). Productivity is largely dominated by a spring diatom bloom (Hobson, 1983; Sancetta and Calvert, 1988) followed by a series of periodic and less intense summer blooms (Takahashi et al., 1977). The composition of settling particles during the summer is dominated by flocs with diatoms, often with intact chloroplasts. During the winter, sinking materials are composed primarily of detrital particles (Sancetta and Calvert, 1988) originating from the Cowichan and the Fraser Rivers outside the fjord (Stucchi and Whitney, 1997).

The absence of macrofauna and the consequent lack of bioturbation in the anoxic sediments leads to the formation of alternating detrital and siliceous layers that represent annual varves. Darker laminations correspond to the input of detrital materials associated with river discharges during the rainy winter period. Light bands are related to the deposition of siliceous diatom frustules during the spring and summer blooms.

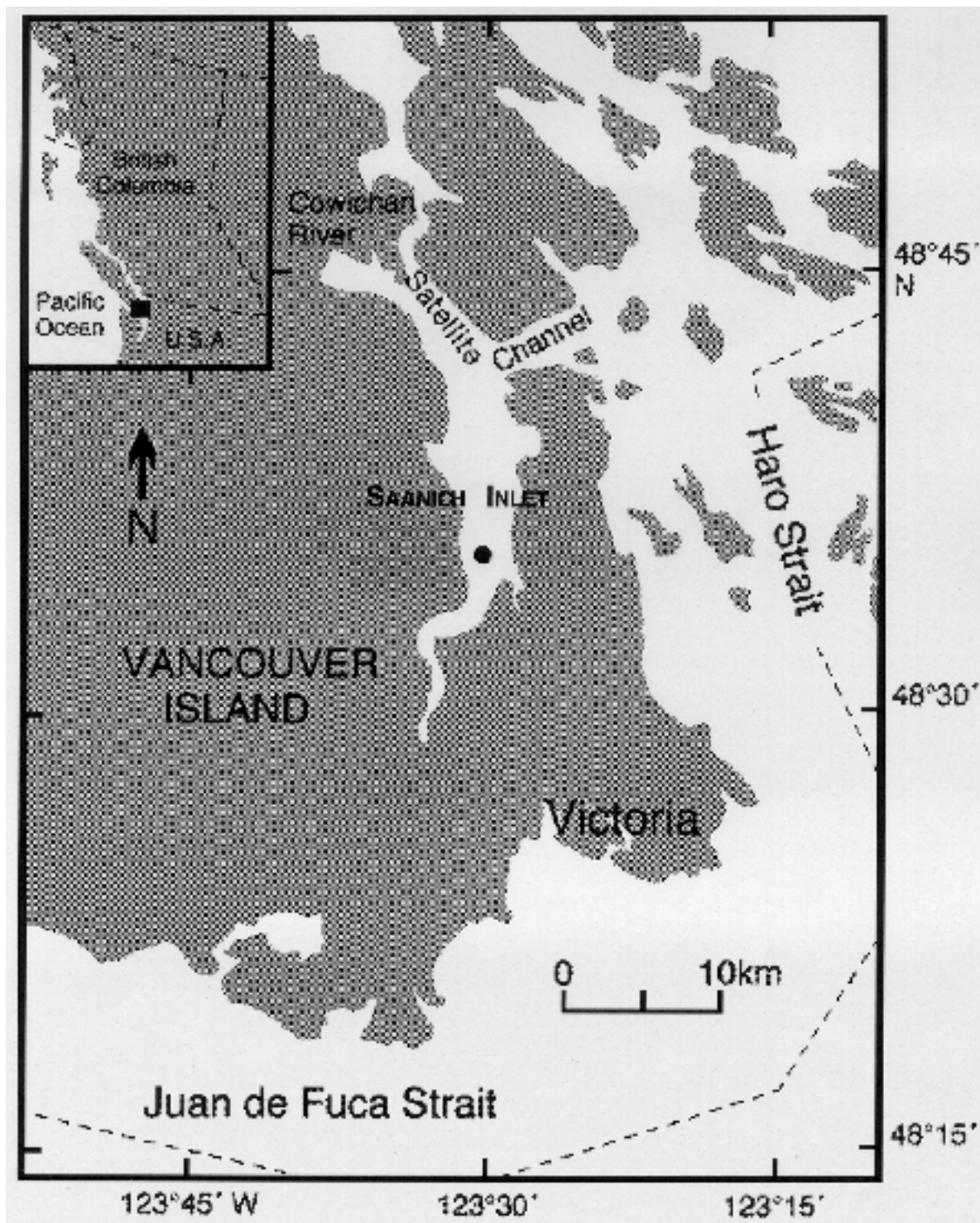


Figure 1. Study area, Saanich Inlet, British Columbia. Dot indicates sampling site at 48°35.1'N, 123°29.5'W.

## **Sample Collection**

Saanich sediments have a high water content, are extremely unconsolidated ("soupy"), and contain methane. These characteristics make collecting sediment cores with intact annual laminations difficult or impossible using conventional techniques such as box coring or gravity coring. For this study we used a freeze-core technique (Shapiro, 1958) modified by Crusius and Anderson (1991). A rectangular aluminum tube filled with a mixture of dry ice and ethanol was inserted into the sediment and left for 20 minutes. The corer freezes the sediment *in situ* prior the retrieval of the corer so that the sediment can be easily retrieved with its laminated structure preserved. We obtained a 145-cm frozen sediment core overlain by frozen seawater indicating that the sediment-water interface was obtained. X-radiographs of the core indicated a laminated structure that corresponded to interbedded bands with high and low detrital content.

The core chronology was established by counting the varves on the X-radiographs. Every light-dark couplet was counted as one year, and we assumed that the first dark layer corresponded to winter 1996–97. The 145-cm core represented 157 years, dating from 1997 to 1840. The uncertainty in the counting was estimated to be  $\pm 2$  years at 100 years. The averaged varve thickness was  $0.85 \text{ cm} \pm 0.4 \text{ cm}$ . Due to sediment compaction, the upper 15 varves showed an uniform decrease in the varve thickness with depth decreasing from 3 to 0.9 cm. Varves corresponding to 1996, 1973, and 1920 were unusually thick (6.1, 2.8, and 5.8 cm, respectively), corresponding to massive layers deposited by subaqueous debris flows (Blais-Stevens et al., 1997). In order to study annual changes in the accumulation of chlorophyll pigments we sampled the sediments at an annual resolution corresponding to each varve.

## **Sample Extraction and Analysis**

Wet sediment samples (approximately 3 g) were weighed into glass test tubes, centrifuged and the overlying water was discarded. Pigments were extracted three times with HPLC grade acetone with sonication in a water bath at  $4^\circ \text{C}$ . The sediments were dried at  $60^\circ \text{C}$  overnight to obtain the dry weight and percent water. The combined solvent extracts were evaporated under an  $\text{N}_2$  stream to a final volume of 4 ml. To purify the extract, 2 ml of 10% aqueous NaCl were added and the resulting mixture was extracted with HPLC grade ethyl acetate until complete decoloration. Samples were dried with anhydrous  $\text{Na}_2\text{SO}_4$ , and the solvent evaporated under an  $\text{N}_2$  stream. Extracts were redissolved in 4 ml of HPLC grade acetone for instrumental analysis.

A Waters 510 liquid chromatograph equipped with an automatic injector (Waters 717+) and a photodiode array detector (190–700 nm, Waters 996) was used to separate and identify individual compounds. The detector was operated at 665 and 410 nm for selective monitoring of chlorophyll derivatives. Separation was performed on a  $\text{C}_{18}$  column (Econosil,  $5 \mu\text{m}$  particle diameter,  $250 \times 4.6 \text{ mm}$ , Alltech Associates Inc.) using a modification of the method described by Zapata (1987). Sample aliquots of  $150 \mu\text{L}$  were mixed with  $50 \mu\text{L}$   $0.5 \text{ M}$  ammonium acetate in water prior to analysis. The elution gradient was programmed from 100% solvent A (80% MeOH and 20% of  $0.5 \text{ M}$  ammonium acetate in water) to 100% solvent B (50% MeOH and 50% acetone) for 20 minutes followed by an isocratic elution for 30 minutes. The solvent flow was  $1 \text{ ml/min}$  for the first 29 minutes and  $2 \text{ ml/min}$  for the rest of the analysis. A pigment extract was analyzed by LC-MS and MS-MS for definitive identification of chlorophyll and its derivatives. Each compound was identified as described in Table 1. Additional details of this analysis will be published elsewhere (Villanueva, in preparation).

The extinction coefficient used for the quantification of chlorophylls and its phaeoderivatives are listed in Table 1. A similar molar absorbtion coefficient for all phaeoderivatives is assumed on the basis that they possess the same chromophore ring. The molar absorbance coefficient for chlorophyllide *a* and pyrochlorophyll *a* has been assumed to be the same as chlorophyll *a*. Since pyropheophorbide *a* and chlorophyllone coelute, they have been quantified together.

Table 1. Chlorophyll pigments quantified by HPLC in Saanich Inlet sediments.

	Compound	Retention time (min)	Absorbance maxima (nm)	Extinction coefficient <sup>1</sup>	Source <sup>2</sup>
1.	Chlorophyllide <i>a</i>	13.8	432/665	100.7 x 10 <sup>3</sup>	a
2.	Phaeophorbide <i>a</i>	18.9	410/665	66.8 x 10 <sup>3</sup>	a
3.	Phaeophorbide <i>a'</i>	19.6	410/665	66.8 x 10 <sup>3</sup>	
4.	Pyropheophorbide <i>a</i>	21.5	410/665	66.8 x 10 <sup>3</sup>	
5a.	Chlorophyllone <i>a</i>	21.1	410/665	66.8 x 10 <sup>3</sup>	
5b.	Chlorophyllone <i>a</i>	21.6	409/668	66.8 x 10 <sup>3</sup>	
6.	Chlorophyll <i>a</i>	26.9	433/665	100.7 x 10 <sup>3</sup>	b
7.	Chlorophyll <i>a'</i>	27.3	433/665	100.7 x 10 <sup>3</sup>	b
8.	Pyrochlorophyll <i>a'</i>	27.7	410/665	100.7 x 10 <sup>3</sup>	c
9.	Phaeophytin <i>a</i>	30.1	410/665	66.8 x 10 <sup>3</sup>	c
10.	Phaeophytin <i>a'</i>	30.5	410/665	66.8 x 10 <sup>3</sup>	c
11.	Pyropheophytin <i>a</i>	32.3	410/665	66.8 x 10 <sup>3</sup>	c
12.	Sterylpyropheophorbide <i>a</i>	35-41	410/665	66.8 x 10 <sup>3</sup>	

1. Molar extinction coefficient at 665nm (cm<sup>2</sup>/M) from Brown (1968). Phaeophytins and phaeophorbides were assumed to have the same molar extinction coefficient.

2. Compounds have been obtained from: a) culture of *Phaeodactylum tricornutum*; b) commercially available; c) synthesized from chlorophyll *a*.

## Results and Discussion

### Qualitative Analysis

The chromatographic profile obtained from a typical Saanich Inlet sediment sample shows the presence of at least thirteen individual compounds that absorb at 665 nm (Figure 2). The dominant peak in each sample is unaltered chlorophyll *a* (compound 6). The other compounds identified are the result of one or several transformation processes of chlorophyll *a* including (Table 1, Figure 3):

1. the loss of the central Mg atom (compounds 2, 3, 4, 5, 9, 10, 11, 12);
2. the loss of the C<sub>13</sub> methoxycarbonyl moiety (4, 8, 11, 12);
3. the loss of phytol moiety (1, 2, 3, 4, 12).

Demetallation of chlorophyll *a* results in the formation of phaeophytins and phaeophorbides. The presence of pyropheophorbide *a*, pyrochlorophyll *a*, and pyropheophytin *a* are the result of the loss of the C<sub>13</sub>-COOMe moiety from the non-pyro-homologue. The loss of the phytol group results in the formation of chlorophyllide *a* and phaeophorbides. Chlorophyllide *a* is usually related to the activity of chlorophyllase, an enzyme contained in diatoms (Jeffrey and Hallegraeff, 1987). These chlorophyll derivatives have been described in senescent algal cultures, phytoplankton blooms, in fecal pellets, in sediment traps, and in surficial sediments. The lack of significant amounts of other derivatives such as mesoderivatives and phorbins (excluding chlorophylls c<sub>1</sub> and c<sub>2</sub>) confirms that pigments are at a very early diagenetic stage in this sedimentary environment.

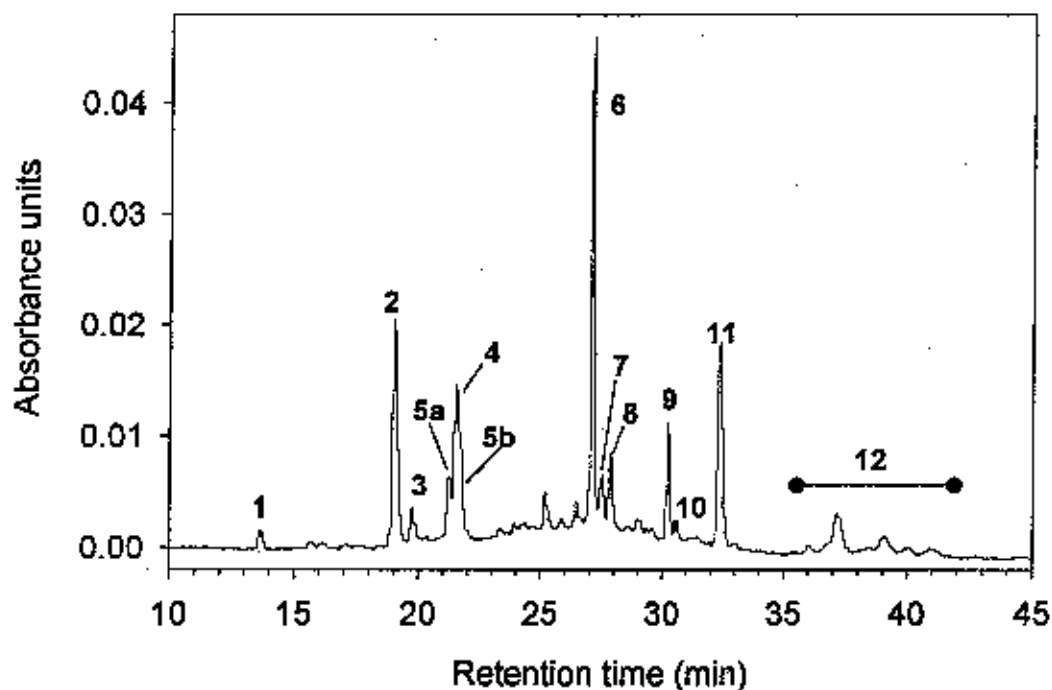


Figure 2. Chromatographic profile obtained from a typical Saanich Inlet sediment.

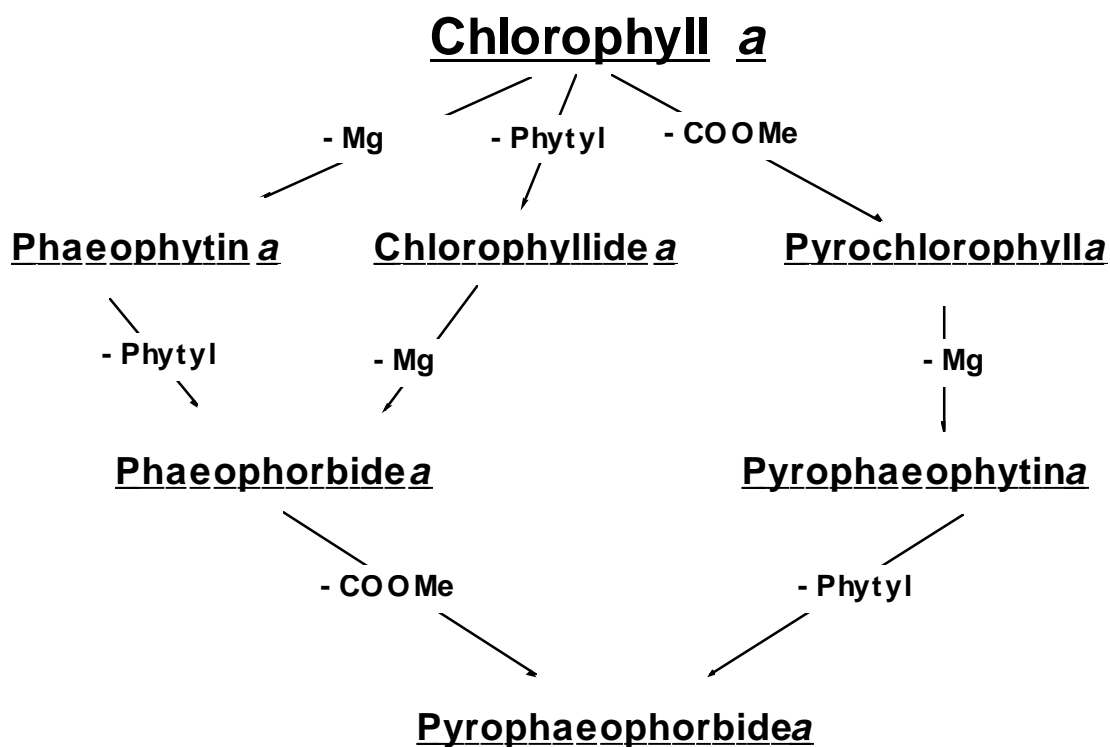


Figure 3. Transformation processes of chlorophyll, and nomenclature for resulting chloropigments.

## Quantitative Analysis

The concentration profiles of the 8 major chloropigments present in Saanich Inlet are shown in Figure 4. The main features of these profiles are 1) the lack of a monotonic downcore change in any of the chlorins; and 2) that different compounds have distinct downcore patterns. Given the lability of these compounds in most depositional environments, the lack of a consistent downcore change in any of the chlorins is remarkable.

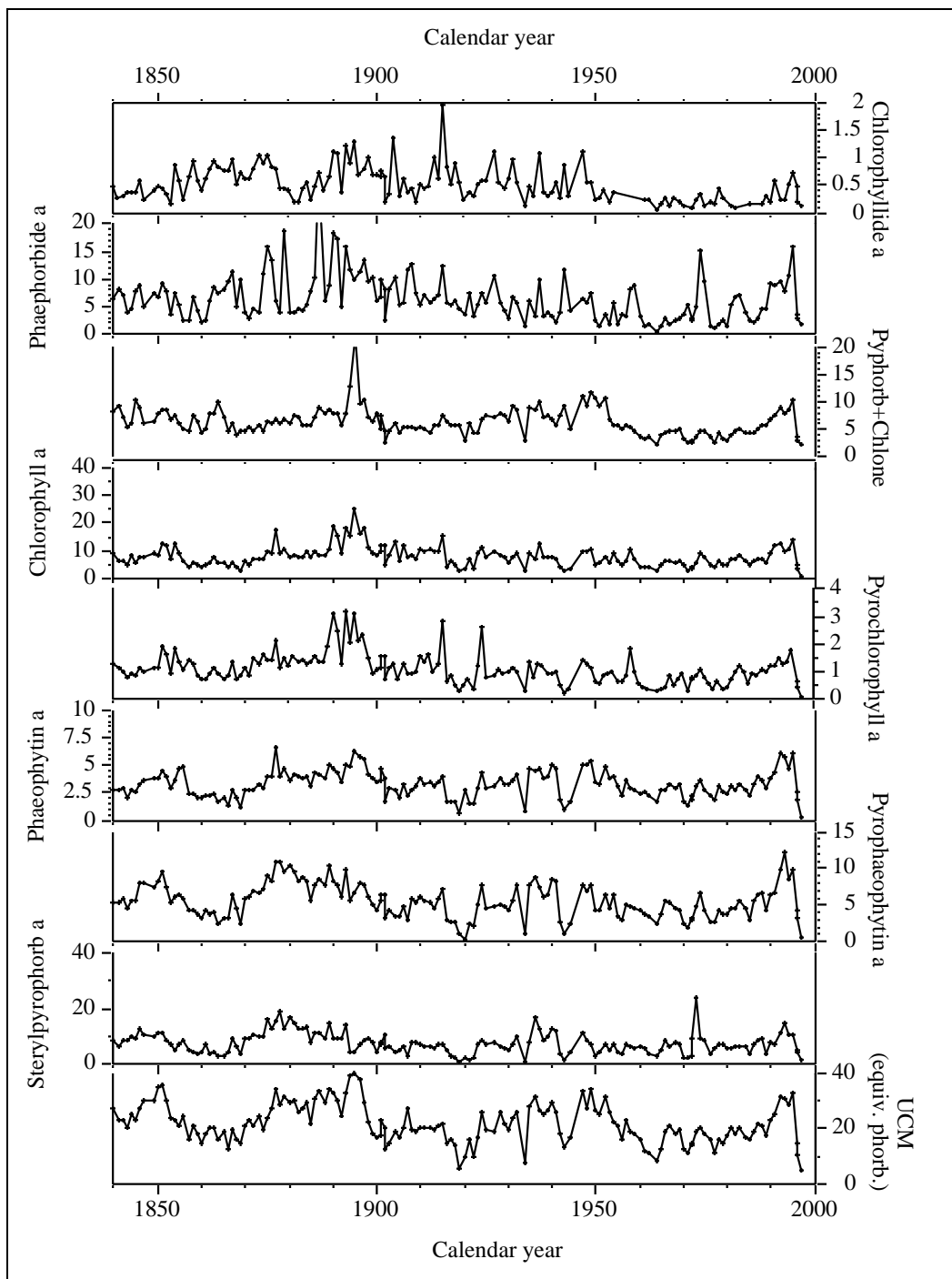


Figure 4. Concentration profiles of chloropigments from annually laminated sediments of Saanich Inlet.

The average composition of samples in the core (Figure 5) offers information on the relative concentration of chlorophyll *a* and its degradation compounds. Chlorophyll *a* represents 20% of the individual compounds detected in Saanich Inlet sediments. The relative contribution of each diagenetic compounds is similar (14–20%) with the exception of chlorophyllide *a* (1%) and pyrochlorophyll *a* (3%) which are the only chlorophyll derivatives that have not undergone demetallation (loss of Mg).

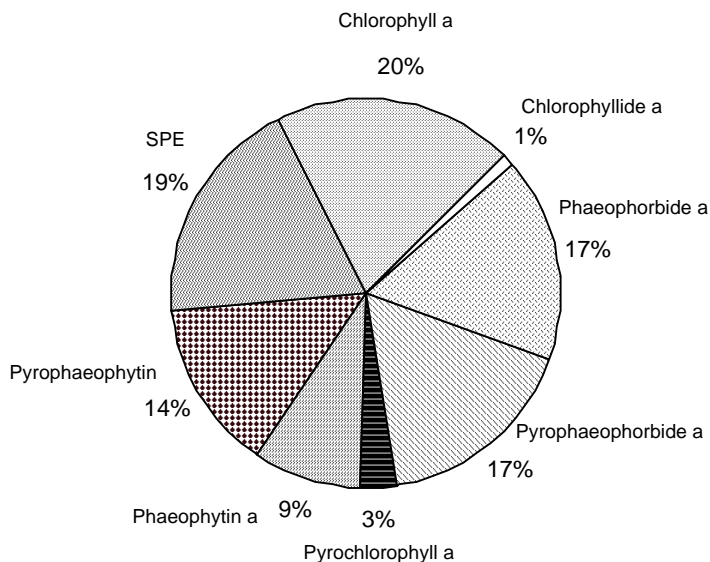


Figure 5. Average composition of chlorophyll pigments in the top 1.4 m of Saanich Inlet sediments.

### **Downcore Diagenetic Trends**

The lack of a consistent downcore trend in individual or total pigments demonstrates that no degradation of chloropigments occurs in the top 1.4 m or 157 years of Saanich Inlet sediments (Figure 4). This feature indicates that processes involving breakdown of the phorbins macrocycle to colorless compounds are not significant in this sedimentary environment over this time interval. The presence of chlorophyll degradation compounds in the sediments is related to processes that take place in the water column, namely respiration in the photic zone and degradation at the sediment-water interface.

As previously mentioned, free chlorophyll *a* undergoes three primary diagenetic reactions: demetallation, loss of the C<sub>13</sub>-COOMe moiety and hydrolysis of the phytol chain. In order to estimate the extent to which each of these degradation reactions occur in very early diagenesis, we calculated the percent of total free compounds that have undergone each degradation process. Demetallation appears to be the preferential process, since it involved an average of 75% (±6%) of the total compounds (Figure 6). The remaining 25% include mostly intact chlorophyll *a* as well as minor amounts of chlorophyllide (1%) and pyrochlorophyll *a* (3%). The formation of pyroderivatives includes 53% (±8%) of the individual compounds while the hydrolysis of phytol occurs less extensively and involves only 34% (±11%) of the total compounds extracted from sediments. The lack of a downcore monotonic trend suggests that these reactions occur before incorporation into the sediments.

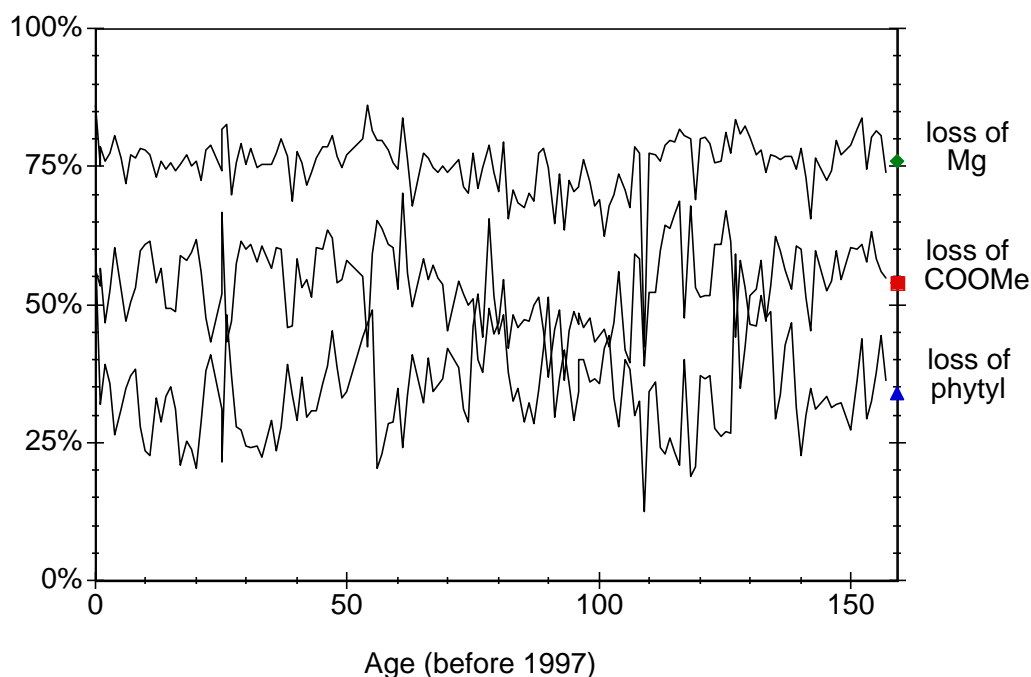


Figure 6. Percent of chloropigments that have lost the central Mg atom (solid line), the methoxycarboxy moiety (dashed line) and the phytol group (dotted line).

### Total Chlorins as a Productivity Signal

The total chlorin concentration in marine sediments has been proposed as a proxy for changes in marine production over glacial-interglacial time scales (Summerhayes et al., 1995; Harris et al., 1996; Schubert et al., 1998). Assuming that there is no change in the rate or extent of degradation we can infer that differences in the pigment concentration are the result of variations in the annual flux that reaches the sediments. Accordingly, the observed changes in the total amount of sedimentary chlorins can be interpreted in terms of temporal variations in production. The coincidence of the published profiles of total chlorins with other productivity proxies such as opal (Harris et al., 1996), other algal lipids (Summerhayes et al., 1995; Schubert et al., 1998), and total organic carbon (Summerhayes et al., 1995; Harris et al., 1996; Schubert et al., 1998), support these interpretations. Since diagenetic processes do not affect pigment composition within the top 1.4 m of sediments, total chlorins can be used to trace variations in the delivery of chlorophyll to the sediment, that, in principle, are related to changes in algal productivity.

Partial flushing of the anoxic bottom waters with dense, oxygenated water from outside the basin occurs annually in late summer or fall (Anderson and Devol, 1973). Since chlorophyll is especially sensitive to degradation by dissolved oxygen (Hurley and Armstrong, 1991; Leavitt, 1993; Sun et al., 1993a; Sun et al., 1993b), annual variability in chloropigment concentrations could be due to the extent of bottom-water renewal and bottom-water oxygen concentration. We tried to determine whether degradation by  $O_2$  at the sediment-water interface was a primary control of chloropigments by comparing our annual record of sedimentary chlorins with a compilation of more than 275 deep water (>170 m) oxygen profiles dating back to 1953 (Stuchhi, D., Institute of Ocean Sciences, Sidney, B.C., unpublished data). No correlation was found, which supports the argument that changes in overlying production is a primary control.

Possible changes in the “transport efficiency” of pigments through the water column can dominate the fraction of the total chlorins produced in the photic zone that ultimately reach the sediments, and therefore could completely alter the productivity signal. No relationship could be found between the sedimentary chlorophyll concentration and the relative abundance of its derivatives. For example, the extent to which specific degradation reactions occur, such as loss of Mg, formation of pyro derivatives, or



incorporation into the polymeric fraction, has been shown to remain relatively constant from year to year in spite of five-fold changes in the total concentration. Also, the year-to-year variability in the extent of phytol-hydrolysis is not correlated with total chlorins. Although the lack of correlation with bottom-water oxygen and constancy in the pigment composition argues against degradation as a major control of the accumulation of chlorins in the sediment, its possible influence in the productivity signal cannot be ruled out. Any interpretation in terms of productivity should be done with caution.

Spectral analyses of the chlorophyll and total chlorin profiles, based on the maximum entropy method, show similar features that include two prominent peaks at 0.009 (110 yr) and 0.053 (18.9 yr) cycles/yr (Figure 7). Since the pigment record covers a time span of only 157 years, we cannot rely on the statistical significance of the 110-year cycle. The presence of six cycles with a periodicity of 18.9 years is observed in both the chlorophyll and total chlorin profiles.

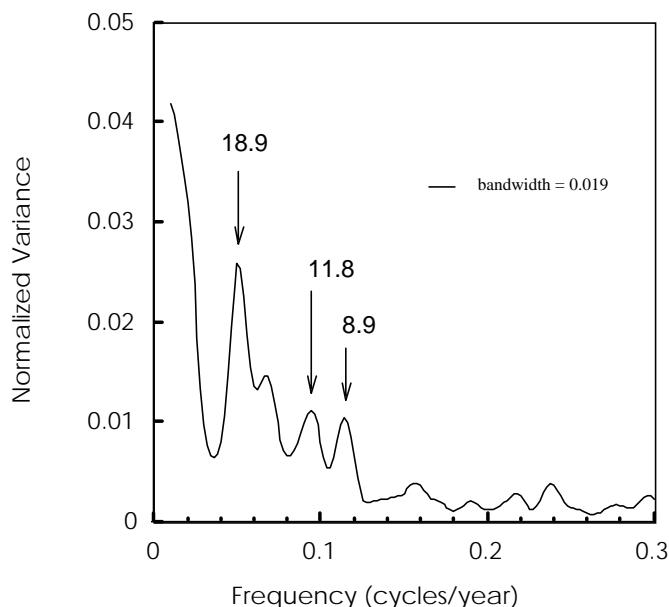


Figure 7. Blackman-Turkey frequency spectrum of the chlorophyll *a* signal in Saanish Inlet sediments over the past 157 years.

Short term (e.g., daily and weekly) changes in production in Saanich Inlet are attributed primarily to the variability in: 1) the tidal mixing outside the mouth of the inlet (Haro Strait) that modulates the input of nutrients to the surface waters of Saanich Inlet; and 2) the stratification of surface waters that favors the development of an algal bloom. Both physical processes are controlled by the spring-neap tidal cycle, giving rise to a biweekly productivity pattern (Parsons et al., 1983; Stucchi and Whitney, 1997). Intensification of tidal mixing results in an increase of the nutrient supply to surface waters, resulting in an intensification of summer blooms. Factors controlling interannual production would include similar processes including tidal mixing (e.g., changes in tidal velocity), fresh water discharge from the Fraser River, density of bottom waters, and insolation. Those factors that are most strongly correlated with changes in the accumulation of chlorins over the past 150 years will be presented elsewhere (Hastings et al., in preparation). The effect of ENSO and the Pacific Decadal Oscillation on each of these factors will allow us to more fully constrain the relationship between climate and productivity in this environment.

## Summary

Chlorophyll is produced by all living plants in the marine environment. Accumulation of this pigment and its transformation products in sediments is potentially a useful proxy for oceanic production. Saanich Inlet presents an unique depositional environment to study changes in overlying production and diagenesis of chloropigments because 1) the laminated sediments allow sampling at an annual resolution with high accuracy; 2) bottom-water anoxia substantially limits chlorin degradation; and 3) overlying production is high and variable. Using a freeze corer, we collected 1.4 m (157 years) of annually laminated sediments without disturbance. Average pigment composition indicates that demetallation is the preferential diagenetic reaction since 75% of the compounds have lost the central Mg ion, followed by formation of pyroderivatives (53%) while hydrolysis of phytol occurs less frequently (34%).

No consistent downcore variation was observed in any of the compounds, total chlorins, or the relative fraction of any one compound or class of compounds. This indicates that no degradation of chloropigments occurs over the 157 year time period represented by the core, and suggests that the transformation products are formed in the water column, or at the sediment-water interface. Since diagenetic processes do not appear to affect pigment concentration or composition, total chlorins can be used as a proxy for changes in primary production. Spectral analysis of the chlorin profile indicates a peak at 0.053 cycles/yr or 18.9 years, suggesting that phytoplankton production in Saanich Inlet has a bi-decadal periodicity.

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